DETERMINATION OF DIAGNOSTIC PREDICTORS OF PROSTATE CANCER AMONG PATIENTS ATTENDING GARISSA COUNTY REFERRAL HOSPITAL, KENYA

JANUARIS MWANTHI MUKAA
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DECLARATION

This research thesis is my original work and has not been presented to any other University.

Signature:……..…..… Date:……...….…….

Januaris Mwanthi Mukaa

- P154/CTY/PT/27471/2019

Department of Medical Laboratory Sciences

SUPERVISORS

We confirm that the work reported in this thesis was carried out by the student under our guidance.

Signature:……..…..… Date:……...….…….

Dr. Wachuka Gathigia Njoroge

Department of Medical Laboratory Sciences

Kenyatta University

Signature:……..…..… Date:……...….…….

Dr. Antony Omondi Radol

Department of Medical laboratory sciences

Kenya Medical Training College

DEDICATION

This thesis project is dedicated to my beloved family and my supervisors who sacrificed their time to review my work and ensured quality in all the processes**.**

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS AND ACRONYMS

BPH	Benign prostate hyperplasia
CTMDR	Centre for traditional medicine and drug research
Conc.	Concentration
DRE	Digital rectal examination
DNA	Deoxyribonucleic acid
EPS	Expressed prostatic secretion
ELISA	Enzyme linked immunosorbent assay
GLOBOCAN	Global cancer observatory
HO1	Null hypothesis
HRP	Horseradish peroxidase
KEMRI	Kenya medical research institute
MRI	Magnetic resonance imaging
NACOSTI	National commission for science technology and Innovation
NPV	Negative predictive value
PPV	Positive predictive value
PAP	Acid phosphatase of the prostate
PHI	Prostate health index scoring
PSA	Prostate specific antigen
PSCA	Stem cell antigen of the prostate
PSMA	Prostate specific membrane antigen
SARDH	Sarcosine dehydrogenase

SEM	Standard error of mean
SPSS	Statistical package for social science
TRUS	Trans rectal ultrasound guided prostate tissue biopsy
W.H.O	World Health Organization
ZPP1	Novel fluorescent sensor for mobile zinc

ABSTRACT

Prostate malignancy refers to a type of cancer that occurs on glandular cells of the prostate gland. It can also occur in other tissues when the cancer cells break away and travel through the blood vessels and lymphoid tissues. The latter is referred to metastatic Prostate Cancer. The disease is prevalent among old men with average age of 60 years. Prostate cancer needs early diagnosis to enable prevention of high mortality and morbidity associated with the disease. Early initiation of treatment is associated with reduced morbidity and mortality hence a need for easily accessible and reliable detection method. Prostatic specific antigen test is nonspecific hence delaying intervention while Confirmatory test (histopathology) is invasive and expensive. The study was aimed at investigating other predictors of prostate cancer in urine that can be an alternative to PSA or be interpreted together with PSA to offer an alternative confirmatory diagnosis instead of histopathology. Such predictors include sarcosine and zinc. Using a cross-sectional study design, all suspected cases of prostate cancer that were found during the study period based on clinical assessment were chosen. In plastic tubes, a volume of roughly 10 milliliters of midstream urine was collected. After centrifuging the urine and collecting the supernatant, zinc and sarcosine levels were measured. The levels of the urinary sarcosine and zinc was compared between study units who developed positive confirmatory test and those whose results are negative for confirmatory test. The collected numerical data was entered into a Microsoft Excel spreadsheet and then imported into the statistical package for social science (SPSS), where it was displayed as the mean, standard error of mean, and +/-standard deviation. The independent t test and analysis of variance (ANOVA) were used to compare the means and standard deviations across different age groups. Predictability of the analytes for the prostate cancer was determined using chi-square. Level of significance was set at 95% i.e., <0.05% for all comparisons. Prostate cancer patients' mean zinc concentration (0.98µmol/ml) was significantly lower than that of control participants (6.20µmol/ml; $p<0.001$). The age groups of prostate cancer patients showed that the zinc concentration used in the calorimeter to diagnose prostate cancer did not significantly differ. ($p = 0.85$). However, among control participants, there was no discernible variation in zinc concentration ($p=0.11$). Additionally, an ELISA test revealed that the sarcosine concentration (4.30±0.11 nmol/ml) in participants with prostate cancer was significantly higher than the control group's (0.47±0.06 nmol/ml) concentration ($p<0.001$). Using ELISA, there was no discernible difference in the age groups of the prostate cancer patients' sarcosine concentration ($p=0.57$). Sarcosine concentrations did not differ significantly between the age groups of the control subjects ($p=0.17$). The ELISA and calorimetric diagnosis results for sarcosine and zinc showed a significant difference between reality and the values for sensitivity, specificity, and predictability ($p<0.001$). The sensitivity of prostate cancer diagnosis using sarcosine ELISA and zinc calorimetric was 93.9% respectively while the specificity for both zinc and sarcosine was 100%. Both zinc and sarcosine recorded a positive predictive value of 100% and a negative predictive value of 93.5%. County governments need to allocate funds for diagnostic facilities to ease the burden of diagnosis. Future researchers should investigate other metabolites to supplement zinc and sarcosine in the diagnosis of prostate cancer.

CHAPTER ONE: INTRODUCTION

1.1 Background information

Prostate cancer refers to a tumor that occurs in the prostate gland. Its etiology is not well understood but there are suggestions that there are contributing risk factors that include high fat diet, low intake of carotenoids, high intake of calcium, hereditary and androgenic factors (Gann PH, 2002). Prostate cancer is of different types the most prevalent being adenocarcinoma representing 99% of all prostate cancers (WHO, 2020). Cancers from the prostate are the most prevalent of all cancers in male population and therefore among the top in the list of death as a result of cancer (Jemal *et al* 2010). In 2020, 1,414,249 new cases of prostate cancer were diagnosed with three hundred and seventy-five deaths reported worldwide (Testa *et al*., 2019). In Africa, cancer of the prostate is a burden recording a high incidence and mortality, with 9.4% new cases and 7.6% deaths (WHO, 2020).

According to report from the Global Cancer Observatory (GLOBOCAN), 887 (8.1%) of all male cancer mortality cases and 1,087 (8.6%) of all male cancer incidence cases in Kenya are estimated to be cases of prostate cancer (Ferlay *et al*., 2020). Cancers of the prostate gland interfere with the homeostasis of prostatic fluid and affect concentration of substances such as zinc and sarcosine (Grayhack *et al*.,1980) Glandular cells in the prostate normally secretes ten times more zinc than other tissues (Lee *et al*., 1980). Secreted zinc levels are extremely reduced with cancer of the prostate when compared to other conditions like benign prostate hyperplasia (Costello & Franklin., 2016). Costello and Franklin (2016) identified a strong relationship between the output of secreted zinc

and cancer, therefore signifying that reduced zinc is a characteristic biochemical marker of prostate cancer. Medarova *et al* (2014) proposed that reduced levels of zinc metabolite in urine could be used for detection of prostate cancer. Kumar *et al* (2020) observed that a number of conditions like benign prostate hyperplasia (BPH), prostatitis, and exercise could raise prostate specific antigen levels. Prostate serum antigen (PSA) can be normal for about 25% of men with prostate cancer leading to false positive (Kumar *et al*., 2013). The definitive test for prostate tumor is prostate tissue biopsy through a Trans rectal (TRUS) or trans perineal method, magnetic resonance imaging (MRI) and ultrasound (Kumar *et al,* 2020). Screening of carcinoma of the prostate involves testing of prostate specific antigen (PSA) and digital palpation of the rectum (Mery *et al*., 2016).

Sensitivity of a test refers to a procedure done to establish how effective a test can identify true positives when compared with a reference test or gold standard that in this study is histology (Bolin E and Lam 2013). Sensitivity= (True Positives (A)/ (True Positives (A)+False Negatives (C). Specificity of a laboratory test refers to procedures done to determine the individuals without a condition or disease when compared with reference test or how well the screening procedure can select the true negatives (Bolin e and Lam 2013) Specificity= (True Negatives (D))/ (True Negatives (D)+False Positives (B)

Predictive value refers to the likelihood of an individual having a condition or disease with results of the test (Parikh *et al*.,2013).

Positive Predictive Value= (True Positives (A))/ (True Positives (A)+False Positives (B))

Negative Predictive Value= (True Negatives (D))/ (True Negatives (D)+False Negatives(C))

The study focuses on determining both the negative predictive values and positive predictive values to establish the probability of study subjects having cancer when biochemically tested for sarcosine and zinc in urine.

1.2 Statement of the problem

In East African countries, prostate cancer has been reported in health facilities but no studies have been done on native populations as reported by globocan 2020 where, prevalence of prostate cancer on a sample size of 23,622 was 7.1%. Incidence reports are expected to rise to over 85% by 2030 (Kumar *et al*., 2020). Mitigation measures that include accessible and reliable screening methods are needed to reduce the mortality cases associated with prostate cancer. According to report from the Global Cancer Observatory (GLOBOCAN), 887 (8.1%) of all male cancer mortality cases and 1,087 (8.6%) of all male cancer incidence cases in Kenya are estimated to be cases of prostate cancer (Ferlay *et al*., 2020). The influx of Somali refugees from Somalia to Garissa increases the burden of prostate cancer since Somalia does not have functional health systems. Garissa county referral hospital is the only public health facility in Garissa County that screens for prostate cancer. The current policies on cancer prevention in Kenya have provided a progressive guidance by giving both legal and frameworks of implementation for the delivery of cancer services in both national and county levels. There are gaps in the implementation of this policy which are lack of enough financing of

cancer services, little or no research and data to enhance the policy and cancer awareness and services emphasized and concentrated in urban areas. There is need for research on a non-invasive diagnostic method, sensitive and suitable for nomadic communities such as Somalis in Garissa County. Screening and early diagnosis of prostate malignancy is the most effective intervention tool for prostate cancer. Therefore, this study focuses on investigation of an alternative screening method to prostate specific antigen (Magoha & Ngumi, 2019). The screening procedure that tests for biochemical indicators of tumor of prostate in urine can supplement prostatic serum antigen in prostate malignancy screening. Variability of sarcosine and zinc in urine among different age groups was determined to show their distribution. Sensitivity, specificity and predictability of prostate cancer was also established for the results of sarcosine and zinc among the different study units to ensure accuracy.

1.3 Justification of the study

The accessibility of prostatic cancer testing by different populations in Kenya and East Africa in general is low (Koitsalu *et al*., 2018). Diagnostic prostate specific antigen (PSA) and Biopsies are not easily accessible in Garissa since only few facilities at all levels perform the tests. The tests are expensive and most of the people living in Garissa are low-income earners hence cannot access diagnosis therefore, there is need to explore other predictors which in this study is sarcosine and zinc secretions into urine. Garissa county referral hospital is the only public health hospital which screens for prostate carcinoma in the whole county. This therefore necessitates for screening method, which is accessible to sub county health facilities and some health centers. The current study is

to investigate diagnostic value of urinary Zinc and sarcosine. If strong association is established with prostate cancer, further development for adaptation to paper strip for use can be done at level two, level three facilities and even mobile clinics, thereby enabling better access to screening prostate cancer. Substances coming from the prostate gland as result of prostate secretion mirror the health of the prostate tissue and therefore analysis of these biochemical metabolites can be insightful to the health of the prostate gland (Mery *et al*., 2016). Urine contains such substances and the urinary zinc and sarcosine vary from normal in prostate tumor (Bray *et al*., 2020).

The results would also inform the policy on the diagnosis of prostate cancer. Data obtained in the research will help in guiding on early diagnosis of suspected prostate cancer patients.

1.4 Null hypothesis (Ho)

Ho. There is no significant difference in urinary zinc and sarcosine levels between confirmed prostate cancer and those not confirmed for prostate cancer.

1.5 Objectives

1.5.1 General objective

To evaluate zinc and sarcosine as diagnostic predictors of prostate cancer among confirmed and control participants attending Garissa County referral.

1.5.2 Specific objectives

i. To determine variability of zinc and sarcosine among the confirmed and control participants to the screening of prostate cancer.
ii. To determine the sensitivity of zinc and sarcosine among the confirmed and control participants to the screening of carcinoma of the prostate.
iii. To determine the specificity of sarcosine and zinc among the confirmed and control participants to the screening of prostate malignancy.
iv. To determine the predictability of zinc and sarcosine among the confirmed and control participants to the screening of prostate cancer.

1.6 Significance of the study

The statistical rise in prostate cancer cases indicates that there is a lack of knowledge and that the general public is not being screened with the available tests. This is because; most of these tests are either too expensive or concentrated to urban areas leaving people in rural areas who are the majority in the population. The current study dwells on targeting prostate cancer biomarkers voided in urine as a result of prostatic secretion. These metabolites include sarcosine and zinc. The results presented in this research helps give an insight into the health of the prostate tissue based on different concentrations of the two metabolites in urine of prostate cancer patients and controls.

1.7 Conceptual framework

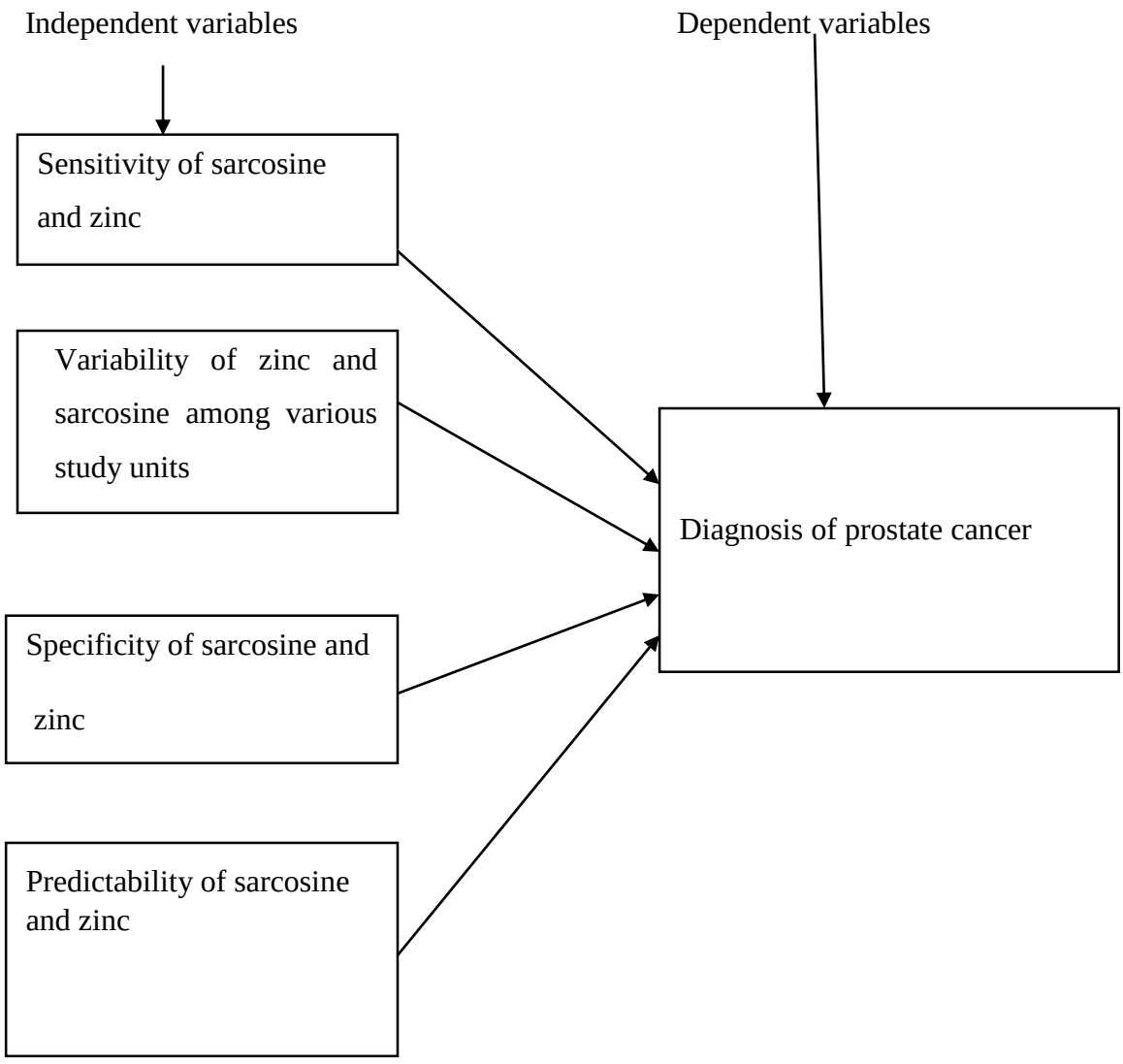

Figure 1.1: Conceptual Framework

The dependent variable is diagnosis of prostate carcinoma. A dependent variable is what a researcher wants to determine in a study (Boyce *et al*.,2012). The independent variables are the factors that determine dependent variables (Alligood *et al*.,1996). The independent variables are the measurements done that affects the dependent variable. The

diagnostic utility are elements comprising of sensitivity, specificity and predictability for the diagnosis of malignancy of the prostate.

CHAPTER TWO: LITERATURE REVIEW

2.1 Prevalence and laboratory testing of prostate cancer

Prostate malignancy alludes to a type of cancer that occurs on glandular cells of the prostate gland. It is the most prevalent of all cancers in men (Zhang *et al*., 2008). Worldwide cancer reports indicate that malignancy of the prostate is the most prevalent of all carcinoma cases (Ervik *et al*., 2014). The global cancer mortality and prevalence reports shows that, 7.3% new cases and 3.8% deaths were reported in the year 2020. According to a study by Bray et al (2020) prostate cancer is the second leading cause of mortality after breast cancer.

Prostate tumor in Europe is the third most diagnosed malignancy and accounting to 24% of all new cancer cases **(**Bray *et al*.,2018). Tumor of the prostate is the second in the United States accounting for 9.5% of all new cancer cases (Capelli *et al*., 2018). Black men from America have high likelihood of having cases of prostate malignancy, as compared to whites (Yamoah *et al.*, 2022).

In Asian populations, Prostate tumor incidence and mortality have increased, but are lower than in Asian-American populations (Ferlay *et al.,* 2020). Although genetic and environmental factors, can partly explain the differences, Lack or inadequate screening of Prostatic specific antigen in Asian subjects is of great influencing reason (Roine *et al.*, 2014).

Prostate cancer tops among the most prevalent cancers in Africa. It ranks third in both incidence and mortality with 9.4 % new cases and 7.6% deaths (W.H. O, 2020). Prostate malignancy is the leading cancer in sub-Saharan Africa (SSA) as far as mortality and incidence cases are concerned. In South Africa, the prevalence of prostate cancer is 8.5% and 3.7% among Black and White men respectively (Roine *et al*.,2014). Prostate cancer prevalence in.

In East Africa, there are expected to be nearly 22 million new cases annually by 2030, up from 14 million in 2012, while the number of deaths is expected to rise from 8.2 million in 2012 to 13 million in 2030, according to a report published in 2017 by the International Agency for Research on Cancer. With an incidence of 39.6%, Uganda has one of the highest rates of prostate tumors seen in Africa. Prostate cancer prevalence in Tanzania stands at 8.8%. According to Krishnan and Truong (2012), awareness on prostate malignancy and screening services is minimal among men in Tanzania.

According to report from the Global Cancer Observatory (GLOBOCAN), 887 (8.1%) of all male cancer mortality cases and 1,087 (8.6%) of all male cancer incidence cases in Kenya are estimated to be cases of prostate cancer (Ferlay *et al*., 2020).

Early screening of cancers of the prostate while localized in the capsule is important since it is curable at the localized stage (Varma *et al*., 1988). Alternative tests that are easy to perform and targeting mass screening of the population are therefore a critical need; hence, biochemical analysis of sarcosine and zinc in urine is an option in this study.

Pathogenesis as well as etiology of prostate cancer has remained idiopathic but Walther *et al.,* (2019) have come with several suggestions including but not limited to; family history, deficiency of vitamin D, high calcium diet, high fat diet, high concentration of sex hormones (androgens), low intake of carotenoids and vasectomy (Walther *et al*., 2019). Adenocarcinoma also referred to as glandular prostate cancers are the most prevalent making up to almost 99% of all prostate cancers. Some types of the cancers such as neuroendocrine do not secrete prostate specific antigen (PSA) and require alternative methods of detection therefore the need for other methods of diagnosis such as urinary zinc and sarcosine.

2.2 Screening of prostate cancer

Early screening of cancers of prostate increases the chances of cure rate (Young, 2002). Some screening procedures for prostate cancer have been discovered and includes, prostatic acid phosphatase (PAP), prostate specific antigen (PSA), prostate specific membrane antigen (PSMA) and prostate stem cell antigen (PSCA). The discovery of prostate phosphatase test as a screening method for tumor of the prostate was done in 1938 by Gutman. Subsequent studies discovered correlation between activity of prostatic acid phosphatase with prostate tumor progression in suspected individuals with prostatic malignancy and therefore prostatic acid phosphatase (PAP) was acknowledged to be a predictor of prostate cancer and treatment (Gutman,1938). Later on, prostatic acid phosphatase (PAP) was replaced by prostatic specific antigen (PSA) which was discovered to be more superior to prostatic acid phosphatase and was adopted to be standard test until today (Wang *et al*.,1979).

However, PSA is non-specific and therefore it has limitations for contributing to over-diagnosis and over-treatment hence controversial in its use for screening of prostate cancer. According to Veeramani *et al*., 2018 the accuracy of prostatic specific antigen in predicting results from treatment and decision making in therapies is not reliable. Therefore, there is urgent need in developing diagnostic methods, which are non-invasive and more specific to supplement PSA (Jamaspishvili *et al*.,2010). Increased prostate specific antigen levels are not exclusive of hyperplasia like BPH, prostatitis, and digital examination of the rectum and therefore as a diagnostic tool lack specificity (Dvorácek *et al*., 2017). Further the biopsies coming from results of prostatic specific antigen test that later give negative results causes unjustified injury to the patients (Mery *et al*., 2016).

2.3 Biochemical markers for prostate cancer

Urine contains substances from metabolism. Substances from circulation are filtered in the bowman's capsule of nephrons into the urinary bladder then voided into urine. These metabolic wastes include both small and large proteins and cells originating from urogenital organs. Various substances from the prostate gland can also be voided together with urine due to its proximity to the urethra (Prensner *et al*., 2012). Deposits in urine are usually separated by centrifugation. The supernatant is rich in biochemical materials including sarcosine and zinc, cell-free nucleic acids, exosomes, glucose and soluble proteins (Elmanoudi *et al*., 2014). Blood tissue comes from virtually all body organs and therefore this makes urine as a source of biochemical markers since it does not have to cross blood tissue barriers. Urine also contains metabolites secreted direct from the prostate gland without interferences and therefore mirrors the health of the prostate

tissue. Urine is non-invasively collected hence easy to analyze secreted prostate fluid. The materials voided in urine from the prostatic secretion have been reported to mirror the health of prostate gland (McCallum *et al*., 1988). Biochemical analysis of urine always needs less processing procedures and the results can be released promptly i.e., the turn-around time of urine tests is less compared to other specimens like blood hence generating a possibility for point-of care-testing when sarcosine and zinc are analyzed for prostate cancer.

Citrate voided in urine is associated with kidney stones (Welshman SG and McGeown, 1976). Spermine detection is complicated because of its electro-optical inactive nature. It reacts with phosphates and sulphides to form macrocytic products hence electro sensors must be employed (Roehrborn, 2005).

2.4 Specificity of secreted components of prostatic fluid

The prostate glands glandular cells secrete large amounts of zinc, ranging from 3000 to 5000 nmols/gram, ten times more than other bodily tissues (Oliver *et al.,* 1980). Other bodily tissues only secrete 200–400 nmols/gram of zinc. Prostate cancers typically impact this biological mechanism, which in turn affects the homeostatic regulation of prostate fluid (Costello & Franklin *et al.,* 2009). Numerous researches have demonstrated that prostate tissue has lower zinc levels than that of BPH and normal subjects (roughly 60–80%) (Costello *et al.,* 2016). Several other studies have confirmed increased levels of zinc in benign prostate hyperplasia (BPH) when compared to normal tissue (Sapota, *et al.*,2009). Virtually all mammals have a zinc concentration of about ten times in the

prostate tissue compared to other body tissues. Organic zinc from other tissues is majorly eliminated in intestine majority of which is absorbed and eliminated in feces but inorganic zinc is excreted in urine (Costello *et al.,* 2016). Decreased urinary zinc levels have strongly been associated with secretory zinc output and therefore this shows that, low urinary zinc level is a characteristic of tumor of the prostate (Medarova *et al.*, 2014). The difference between the current study and the study done by Eskra et al. (2013) about the role of zinc in urine in prostate cancer diagnosis is that, zinc quantification was done using zinc sensors detected using fluorescent spectrophotometer whereby the zinc scores were achieved by multiplying zinc concentration in micromoles with the concentration of creatinine in micrograms per ml as opposed to the current study which used calorimetric technique.

Metabolism of the prostate tissue leads to the release of prostate fluid that contains zinc, sarcosine, spermine, citrate and other substances (Kelly *et al.,* 2016). N-methyl glycine also called sarcosine, was discovered by Justus from German in the year 1847. It is an intermediate product of glycine amino acid formed as a result of synthesis and degradation (methylation) of glycine (Laxman *et al.,* 2009). It is present in the body in trace amounts. Sarcosine dehydrogenase, glycine N-methyltransferase, and L-pipecolic acid oxidase are the primary enzymes that regulate sarcosine metabolism (Kerr, 1972). Cells release S-adenosylhomocysteine when a methyl group from S-adenosylmethionine is chemically transferred to glycine, producing scosine (Mukherjee *et al.*, 2012).

The chemical reaction is mediated by N-methyltransferase which is released with raised levels in the liver, pancreas, and prostate tissue. This study is targeting sarcosine generated from the prostate tissue which is voided in urine.

Sarcosine is found in high concentrations in blood plasma and urine in prostate cancer (Goldstein *et al*., 2012). Sarcosine has been discovered to enhance development of malignant cancer cells on the prostate tissue hence can act as a good predictor in the diagnosis of prostatic malignancy when detected in urine (Sreekumar *et al.,* 2009). Secretory output levels of sarcosine from glandular cells of the prostate into urine are raised in cancer of the prostate and therefore can distinguish between benign hyperplasia and prostate malignancy. Screening of prostate cancer using biochemical urine analysis for testing of sarcosine and zinc can supplement prostatic specific antigen in the screening of malignancy of the prostate tissue (Sreekumar *et al*., 2009).

2.5 Predictability when screening for prostate cancer

In predicting prostate malignancy, Rotterdam arm of the European Study proposed a model data for Screening tumor of the prostate tissue with an elevated discrimination value when compared with prostate specific antigen (Jansen *et al*.,2012). The dynamics of quantitative analysis of cancer of the prostate under treatment may be affected by the unavailability of identifiable parameters from the available data hence limiting the predictive ability of the model (Roobol., *et al*., 2013). The negative predictive values and positive predictive value were determined to establish the likelihood of study subjects having cancer when biochemically tested for sarcosine and zinc in urine.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study design

The research utilized a cross-sectional design with an adjusted participant count based on Cochran's formulae, as determined by Fischer *et al.* (2006). Based on histopathological findings, the levels of zinc and sarcosine in men with confirmed prostate cancer were compared to those in men without confirmed prostate cancer.

3.2 Study site

The research site was Garissa County referral hospital. It is located approximately one kilometer from Garissa town. It is the only level five facility in the county that offers specialized testing of prostate cancer. Most of health facilities are inaccessible due to poor road networks and therefore leading to health burden of diseases and conditions to the local population who are the majority in Garissa and northeastern province at large. Biopsy samples are referred to facilities in Nairobi and this signifies the constraints faced by patients since they are required to pay huge cash for the biopsy procedure and histopathology. Majority of people in Garissa are nomads living in rural set up far away from the referral health facility therefore the study on predictors of prostate cancer in urine will provide a reprieve to the nomadic communities.

3.3 Study population

Target population included all the confirmed positive prostate cancer participant and confirmed negative prostate cancer participants.

3.3.1 Inclusion criteria

All the prostate cancer patients presenting with signs and symptoms suggestive of prostate cancer visiting Garissa County referral hospital during the research period were eligible for inclusion in the study upon consenting. The non-prostate cancer suspects who consented participated in the study as prostate healthy controls.

3.3.2 Exclusion criteria

All the study units who did not accept to be part of the study were excluded from participation.

3.4 Calculation of the sample size

Sample size was done using Fischer *et al.,* 2006 method of calculating prevalence where:$n = Z2pq/d2$

Prevalence of prostate cancer in Kenya is 8.6% therefore, P = 0.086, Q =0.914, Z =1.96, d = 0.05. (1.96 x 1.96x0.086x 0.914) / $(0.05)^2$ = 120

This gives 120 study units. The population size (N) is less than 10,000 therefore, the Cochran's formular was modified to:

The researcher employs Cochran's equation in conjunction with a population correction to determine sample size for small populations of known size.

$$n = \frac{n0}{1 + (no - 1)/N}$$

Where; n_0 = Cochran's sample size recommendation (120)

N=Population size (130)

n=New adjusted sample size

$n = \frac{120}{1+(120-1)/130} = \frac{120}{1+(\frac{119}{130})} = 120/1.92$ = 62 participants

3.5 Sampling technique

Based on the reporting rates of patients meeting the criteria for inclusion during one month of pilot study, sampling criteria was determined by systematic sampling technique. The individual participants to be sampled first was determined by dividing the approximate population (130) by the sample size (62) thereby getting positive integer two. Individual samples for prostate cancer patients and controls(negative) were then chosen at regular intervals starting with the second patient until a sample of 31 confirmed positive and 31 negative (control) study units were achieved.

3.5.1 Sample collection and processing

Midstream urine samples were collected from confirmed and those not confirmed of prostate cancer in the oncology clinic-Garissa County Referral Hospital. The rationale of collecting samples from the confirmed patients for prostate cancer is because they are presumed to have high levels of sarcosine metabolites and low concentration of zinc in urine when compared with controls. Urine was collected in urine containers coded from P001 and Pc 001.Urine was collected in clean plastic bottles; this is because siliconized rubber stoppers of commercial control sera containers may contain zinc that can affect results. Solid components of urine from each individual were centrifuged at 1000rv/min for 5 minutes. Supernatant contains the soluble substances that include proteins,

exosomes, biochemical components (zinc, sarcosine). Supernatant urine samples were kept at -80 degrees centigrade awaiting analysis.

3.6 Laboratory analysis

3.6.1 Zinc analysis

Zinc quantification kit from Abcam's company, lot Number GR3415592 manufactured in the year 2022 was used.

3.6.1.1 Test principle for zinc analysis

Zinc concentration in urine samples was detected using zinc detector in which zinc was bound to the probe and then measured calorimetrically at 485nm. The intensity of color formed by the chromophore was directly proportional to the concentration of the zinc ions in the sample.

3.6.1.2 Procedures

The samples and reagents were first placed at room temperature prior to analysis on a bench free of contamination. Eppendorf tubes were then labelled blank 1 and blank 11, standard 1 and negative (control) samples.

3.6.1.3 Preparation of standards and samples

500ul of samples were aliquoted using 500ul pippete to Eppendorf tubes labelled S and Sc. The samples were then centrifuged at 25 degrees centigrade at 3000 revolutions /min for 20mins.

An aliquot of 100ul of sample supernatant was then transferred to fresh clean Eppendorf tubes labelled S and Sc.

Table 3.1: Preparation of standards for zinc analysis

30 µMol	Standard 1	100ul of 100 µM$ZnCl_2$ std +300ul assay buffer
10 µMol	Standard 2	100ul standard 1 +300ul assay buffer
3 µMol	Standard 3	100ul standard 2+ 300ul assay buffer
1 µMol	Standard 4	100ul standard 3+ 300ul assay buffer
0.3 µMol	Standard 5	100ul standard4 + 300ul assay buffer
0.1 µMol	Standard 6	100ul standard5 + 300ul assay buffer
0 µMol	Standard 7	100ul standard6 + 300ul assay buffer

Table 3.2: The concentration of standards

Std conc	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Standard 6	Standard 7
100µMol $ZnCl_2$	30 µMol	10 µMol	3 µMol	1µMol	0.3 µMol	0.1 µMol	0 µMol

The samples were then diluted with 50µl assay buffer

3.6.1.4 Preparation of standards

10µl of 100µMol $ZnCl_2$ standard was diluted into 990µl assay buffer to get 1mM$ZnCl_2$ standard solution (990µl +10µl=1mMol$ZnCl_2$).

100µl of 1mM$ZnCl_2$ standard solution was then added to 900µl assay buffer to get 100µMole$ZnCl_2$ standard solution.

300µl of 100µM standard solution was taken to perform 1:3 serial dilution to get 30,10,3,1,0.3,0.1,0µM serially diluted standards as shown below.

96 well plate diagrams were drawn to give the layout of the analytes from blank, standards and samples in duplicates. The wells were clearly marked for analytes

50µl of $ZnCl_2$ standards, the diluted sample and blank control (distilled water) was then transferred into each well in duplicates.50ul of distilled water was transferred to the blank control well.

The zinc detector was first homogenized in a centrifuge and vortexed to allow proper mixing. 25µl of zinc detector was then added into 5ml assay buffer into a falcon tube (1000µl pippete was used) thus making zinc assay buffer.

Multichannel pippete was used to transfer 50ul of zinc assay buffer into each labelled wells in duplicates i.e., $ZnCl_2$ standard, blank control, and test samples to make the total zinc chloride assay volume 100µl/well. Sealing of the plate was done by use of plate sealer.

The reaction was incubated for 10 mins at room temperature protected from light. The absorbance of each well was immediately determined using microreader plate at 485nm.The results was displayed and used to draw a standard curve.

3.6.2 Sarcosine analysis

Human sarcosine ELISA Kit from MyBioSource company, lot no.202204002 manufactured 2022 was employed.

3.6.2.1 Principle for sarcosine analysis

Analysis of sarcosine employed a sandwich quantitative Elisa assay principle. The plate was precoated with human sarcosine antibody. Sarcosine antigen in the sample was transferred to the wells and attached to the antibodies present in the plate. Antisarcosine antibody was transferred to the mixture and reacted with sarcosine present in the reaction mixture. Horse dish peroxidase enzyme was added. On addition of Substrate, the solution changed color and stop solution was added hence optical density read calorimetrically at 450nm.

3.6.3 Sample and Reagent preparation

All the samples and reagents were first placed on the working bench free on contamination to allow them to thaw at room temperature. Clean Eppendorf tubes (1.5ml) were then clearly labelled.

The samples were centrifuged at 3000r/min for 20mins with centrifuge set at 25 degrees centigrade. Supernatant was transferred to clean Eppendorf tubes labelled S1-S31 and Sc1 to Sc 31.

3.6.4 Standards and blank control preparations

Clean Eppendorf tubes were clearly labelled std5, std 4, std3, std2, std1 in duplicates. Blank control tubes were labelled blank 1 and blank 11 in duplicates.

Standards were reconstituted in the following manner**:**

120ul of standard (16nmol/ml) was mixed with 120ul of diluent and transferred to tubes labelled standard5 generating 8nmol of the stock. 2-fold serial dilution (1:2) was employed the diluent to produce 4nmol/ml, 2nmol/ml, 1nmol/ml and 0.5nmol/ml as shown below.

Table 3.3: Preparation of standards

8nmol/ml	Standard 5	120µl original std+120µl standard diluent
4nmol/ml	Standard 4	120µl std5 + 120µl standard diluent
2nmol/ml	Standard 3	120µl std4 +120µl standard diluent
1nmol/ml	Standard 2	120µl std3 +120µl standard diluent
0.5nmol/ml	Standard 1	120µl std2+ 120µl standard diluent

Table 3.4: Standard concentration of sarcosine

Standard conc	Standard5	Standard4	Standard3	Standard2	Standard1
16nmol/ml	8nmol/ml	4nmol/ml	2nmol/ml	1nmol/ml	0.5nmol/ml

3.6.5 Buffer Preparation

20mls of wash buffer solute was put into 480mls of buffered water in a clean falcon tube to yield 500mls.

It was slowly homogenized to ensure any formed crystals were dissolved.

3.6.6 Procedure

96 well plate diagrams were first drawn to give the layout of analytes i.e., Standards, samples, sample controls and blanks in duplicates. Analysis was performed at room temperature.

50 µl of standard solutions were transferred using multichannel pippete to wells No.5 to No.1 in duplicates. The standard pots contain biotinylated antibody hence was not added.

40ul of samples were transferred to sample wells in duplicates. 10µl of antisarcosine antibody was added to samples. 50µl streptavidin-HRP was reconstituted and added to sample pots, standards and mixed well.

Blanks were prepared by transferring 40ul of distilled water and 10µl of antisarcosine antibody.

All the solutions were mixed and covered with a sealer. Incubation was done at 37 degrees centigrade for 60mins.The plate was then put in a wash buffer and washed five times soaking wells with 300µl wash buffer for 1min after each wash.

The plate was bloated with paper towels after each wash taking care not to suck air bubbles which obscures the binding sites. 50µl of substrate A was added to all wells and 50µl of solution B subsequently added.

The plate was covered with a new sealer and incubated at 37 degrees centigrade for 10 mins in the dark. 50µl of stop solution was transferred to all wells in a chemical hood. The formed color blue instantly changed to yellow. The absorbance of each well was read using microreader plate at 450nm within 10mins after adding stop solution. The standard curve was then generated and used to determine concentration of samples.

3.7 Sensitivity, specificity and predictive values of zinc and sarcosine in diagnosis of prostate cancer

According to the reference standard, the sensitivity, specificity, and predictive values of zinc and sarcosine in the diagnosis of prostate cancer were computed, as shown in table 3.5. The study participants were evaluated on the two screening tests, regardless of the classification that was determined using the reference standard. Whether the test results fall below or above a given cutoff point on a continuum served as the basis for the tests. In assess sensitivity, specificity and predictive values, this study used cutoff of 0.1-

3µmol/ml and 1-8nmol/ml in zinc and sarcosine diagnosis of prostate cancer, respectively.

Table 3.5: Derivation of sensitivity, specificity, and predictive values

		Disease (number	**Non-Disease (number)**	**Total (number)**
Test result	Positive (number)	A (True Positive)	B (False Positive)	Test [Positive
	Negative (number)	C (False Negative)	D (True Negative)	Test Negative
		Disease	Non-Disease	Total

As shown in table 3.5, each individual was assigned to one of the four cells labeled A, B, C, or D based on the reference standard and screening test results depending whether the reference standard indicated the presence or absence of the target condition, and if the screening test produced a positive result (the subject looks to have prostate cancer) or a negative result (the subject does not appear to have prostate cancer). The numbers of individuals in each of the four cells were used to calculate the sensitivity, specificity, and predictive values, which were then expressed as percentages using the following formulas:

$$\text{Sensitivity} = \frac{\text{A}}{\text{A} + \text{C}} \times 100$$

$$\text{Specificity} = \frac{\text{D}}{\text{B} + \text{D}} \times 100$$

$$\text{Positive predictive value} = \frac{A}{A + B} \times 100$$

$$\text{Negative predictive value} = \frac{D}{C + D} \times 100$$

3.8 Data presentation and analysis

3.8.1 Statistical data analysis

The raw data were tabulated in Microsoft Excel before being transferred to the statistical package for social science (SPSS) software version 26 for analysis. For quantitative data, descriptive statistics were expressed as mean, standard deviation, standard error of the mean, range, variance, minimum and maximum value, as well as frequency and percentage in participant characterization. The inferential statistic independent t-test was used to compare zinc or sarcosine concentrations in prostate cancer patients and controls. ANOVA was used to compare zinc or sarcosine concentrations among participant age groups. The significance level was set at $p<0.05$. Tables and graphs were used to present the data.

3.9 Ethical approval and confidentiality

Ethical approval was obtained from the Kenyatta University Ethical Review Committee and the Garissa County Referral Hospital Administration, as well as a Permit to conduct the research from the National Commission for Science, Technology, and Innovation (NACOSTI). Informed consent forms were also made available to all study participants.

CHAPTER FOUR: RESULTS

4.1 Social-demographic characterization of participants

Sixty-two male participants attending prostate cancer screening in Garissa County Hospital participated in this study. Among the participants, 31 were prostate cancer positive, while 31were prostate cancer negative.

4.1.1 Marital status of the participants

Among the prostate cancer positive participants, 96.8% were married, whereas 3.2% of the participants were single. However, high proportion of prostate cancer negative participants were married with a proportion of 96.8%, while the single participants had a proportion of 3.2%.

4.1.2 Residence of the participants

Of the study participants who were prostate cancer positive, 41.9% were urban dwellers, while 58.1% were rural dwellers. The rural residence had a higher proportion of 54.8%, whereas urban residence had a proportion of 45.2% among study participants who were prostate cancer negative.

4.1.3 Occupation of the participants

In this study, the self-employed study participants had highest percentage of 83.9%, followed by employed (12.9%) and unemployed (3.2%) among the prostate cancer positive participants. Among the prostate cancer negative participants, the highest

proportion of 71.0% were self-employed, followed by unemployed (16.1%), while employed had a proportion of 12.9%.

4.1.4 Ethnicity of the participants

This study used participants who were belonging to 7 ethnic groups in Kenya. Of the prostate cancer positive participants, the Somali community had the majority with a proportion of 80.6%, followed by Kamba, Luhya, Kikuyu, Luo and Meru communities with proportions of 6.5%, 4.2%, 3.2%, 3.2% and 3.2%, respectively. Similarly, the Somali ethic group had a higher proportion of the participants who were prostate cancer negative, followed by Kamba, Luhya, Luo, Kikuyu and Oromo communities with proportions of 9.7%, 3.2%, 3.2%, 3.2% and 3.2%, respectively (Table 4.1).

Table 4.1: Social demographic characteristics of study participants

	Study participants	
Variable	**Prostate cancer positive**	**Prostate negative cancer**
Age groups		
<60	4 (12.9%)	15 (48.4%)
60-69	9 (29.0%)	11 (35.5%)
70-79	7 (22.6%)	4 (12.9%)
≥80	11 (35.5%)	1 (3.2%)
Marital		
Status		
Married	30 (96.8%)	30 (96.8%)
Single	1 (3.2%)	1 (3.2%)
Residence		
Urban	13 (41.9%)	14 (45.2%)
Rural	18 (58.1)	17 (54.8%)
Occupation		
Employed	4 (12.9%)	4 (12.9%)
Self-employed	26 (83.9%)	22 (71.0%)
Unemployed	1 (3.2%)	5 (16.1%)
Ethnicity		
Somali	25 (80.6%)	24 (77.4%)
Meru	1 (3.2%)	0 (0%)
Kamba	2 (6.5%)	3 (9.7%)
Luhya	1 (3.2%)	1 (3.2%)
Luo	1 (3.2%)	1 (3.2%)
Kikuyu	1 (3.2%)	1 (3.2%)
Oromo	0 (0%)	1 (3.2%)
Total		

4.2 Calorimetric concentration of zinc and Sarcosine ELISA diagnosis of prostate cancer

The mean, standard deviation, variance, standard error of the mean, range, minimum value and maximum value of zinc concentration in prostate cancer positive study participants were 0.98, 0.72, 0.52, 0.13, 1.97, 0.03 and 2.00µmol/ml, respectively (Table 4.2). On the other hand, the prostate cancer negative study participants recorded a mean, standard deviation, variance, standard error of the mean, range, minimum value and maximum value of zinc concentration of 6.20, 1.45, 2.09, 0.26, 4.03, 5.01 and 9.04 µmol/ml, respectively (Table 4.2). The mean zinc concentration (6.20µmol/ml) of prostate cancer negative study units was significantly higher than the mean concentration (0.98µmol/ml) of prostate cancer positive units ($p<0.001$; Table 4.2 and Figure 4.1).

Table 4.2: Concentrations of zinc among prostate cancer positive and prostate cancer negative participants using Calorimetric method

	Zinc concentration (µmol/ml)	
Descriptive statistics	**Prostate cancer positive**	**Prostate cancer negative**
Mean	0.98	6.20
Std. error of mean	0.13	0.26
Median	0.94	5.36
Std. deviation	0.72	1.45
Variance	0.52	2.09
Range	1.97	4.03
Minimum	0.03	5.01
Maximum	2.00	9.04

Std. = standard; $p<0.05$

Prostate cancer positive participants had the lowest zinc concentration in urine (0.98µmol/ml), whereas prostate cancer negative participants had the highest zinc concentration (6.20µmol/ml). Therefore, zinc concentration levels according to this study can be adopted for screening of cancer patients.

The age groups of prostate cancer positive participants demonstrated that there was no significant difference in zinc concentration in diagnosis of prostate cancer using calorimeter ($p=0.85$; Table 4.3). The age groups of <60, 60-69, 70-79 and ≥80 had zinc concentrations of 1.02±0.36, 1.15±0.27, 0.86±0.23 and 0.89±0.23µmol/ml, respectively (Table 4.3). On the other hand, there was no significant variation in zinc concentration among prostate cancer negative participants ($p=0.11$; Table 4.3). The age groups of <60, 60-69, 70-79 and ≥80 had zinc concentrations of 6.36±0.40, 5.61±0.25, 6.58±0.93 and 8.93±0.00µmol/ml, respectively.

The prostate cancer positive participants had a mean, standard deviation, standard error of the mean, variance, range, minimum value and maximum value of sarcosine concentration of 4.30, 0.60, 0.11, 0.36, 1.81, 3.30 and 5.11nmol/ml, respectively. In contrast, the sarcosine concentration of prostate cancer negative participants had a mean of 0.47 with a standard deviation of 0.35, standard error of the mean of 0.06, variance of 0.12, range of 1.00 and minimum and maximum values of 0.04 and 1.04nmol/ml (Table 4.4). Using ELISA, the mean sarcosine concentration (4.300.111nmol/ml) in prostate cancer positive participants was significantly higher than the concentration (0.470.06nmol/ml) in prostate cancer negative participants ($p<0.001$).

Table 4.3: Zinc concentrations by age groups in prostate cancer positive and prostate cancer negative participants using calorimeter

	Zinc concentration (µmol/ml)	
Age groups	**Prostate cancer**	**Control**
<60	1.02±0.36	6.36±0.40
60-69	1.15±0.27	5.61±0.25
70-79	0.86±0.23	6.58±0.93
≥80	0.90±0.23	8.96±0.00
p value	0.85	0.11

Mean±standard error of the mean along the column had no significant difference when subjected to one-way ANOVA ($p>0.05$).

Table 4.4: Concentrations of sarcosine among prostate cancer positive and prostate cancer negative participants using ELISA

	Sarcosine concentration (nmol/ml)	
Descriptive statistics	**Prostate cancer**	**Control**
Mean	4.30	0.47
Std. error of mean	0.11	0.06
Std. deviation	0.60	0.35
Variance	0.36	0.12
Range	1.81	1.00
Minimum	3.30	0.04
Maximum	5.11	1.04

Std. = standard; $p<0.001$

Prostate cancer positive patients among the different age groups had no significant variation with sarcosine concentration ($p=0.57$;. The age groups of <60, 60-69, 70-79 and

≥80 had sarcosine concentrations of 4.03±0.39, 4.51±0.13, 4.30±0.28 and 4.23±0.19nmol/ml, respectively. Prostate negative participants were not significantly different among the different age groups in sarcosine concentration (p=0.17; Table 4.5). The age groups of <60, 60-69, 70-79 and ≥80 had sarcosine concentrations of 0.57±0.09, 0.34±0.0.09, 0.32±0.16 and 0.92±0.00nmol/ml, respectively.

Table 4.5: Sarcosine concentration by age groups of prostate cancer positive and prostate cancer negative participants using ELISA

	Sarcosine concentration (nmol/ml)	
Age groups	**Prostate cancer**	**Control**
<60	4.03±0.39	0.57±0.09
60-69	4.51±0.13	0.34±0.09
70-79	4.30±0.28	0.32±0.16
≥80	4.23±0.19	0.92±0.00
p value	0.57	0.17

Mean±standard error of the mean was not significantly different as shown above using one-way ANOVA (p>0.05).

4.3 Sensitivity of zinc (calorimetric) and sarcosine (ELISA) in diagnosis of prostate cancer

Objective two was focusing on determining sensitivity of zinc and sarcosine to the diagnosis of prostate cancer. This research utilized a cutoff range of 0.1-3µmol/ml and 1-8nmol/ml to examine sensitivity of zinc and sarcosine respectively. The sensitivity of prostate malignancy using zinc calorimetric method was 93.55%, while the sensitivity of prostate cancer diagnosis using sarcosine ELISA was 100% (Figure 4.3). This study showed that both the calorimetric analysis of zinc and ELISA analysis of sarcosine in

urine had high sensitivity. This therefore shows that the number of false negatives in this study is low, hence can select every individual with prostate cancer.

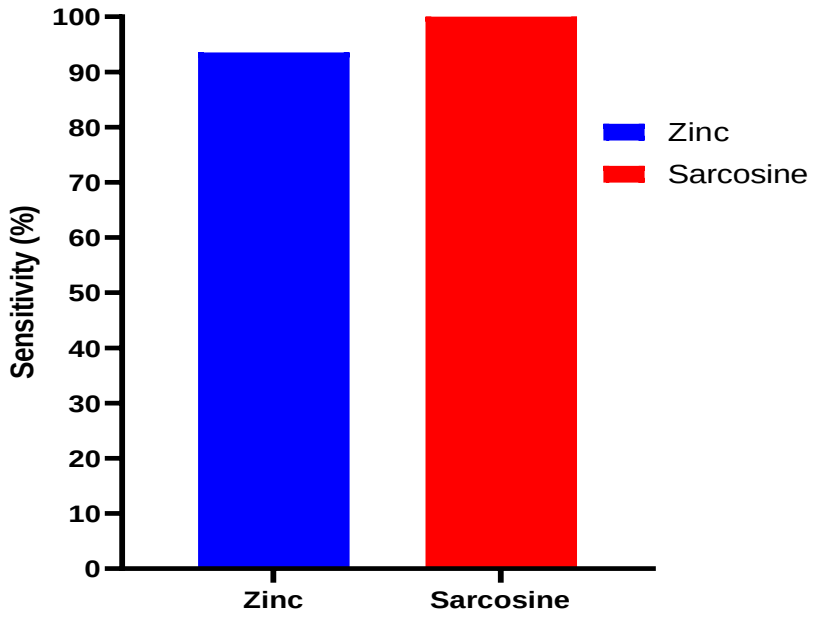

Figure 4.1: Sensitivity of zinc and sarcosine in diagnosis of prostate cancer using Calorimeter and ELISA techniques

4.4 Specificity of zinc and sarcosine in diagnosis of prostate cancer using Calorimeter and ELISA methods, respectively

The third objective which was dwelling on determination of specificity of zinc and sarcosine to prostate cancer diagnosis and therefore a reference standard cutoff of 0.1-3µmol/ml and 1-8nmol/ml was used to examine specificity of zinc and sarcosine respectively. The specificity of prostate malignancy using zinc calorimetric was 100%, whereas the specificity of prostate cancer diagnosis using sarcosine ELISA was 93.55% (Figure 4.4). This study revealed that specificity of zinc and sarcosine was high with both

calorimetric and ELISA methods respectively. The study therefore notes that the two screening methods are able to select individuals without prostate cancer.

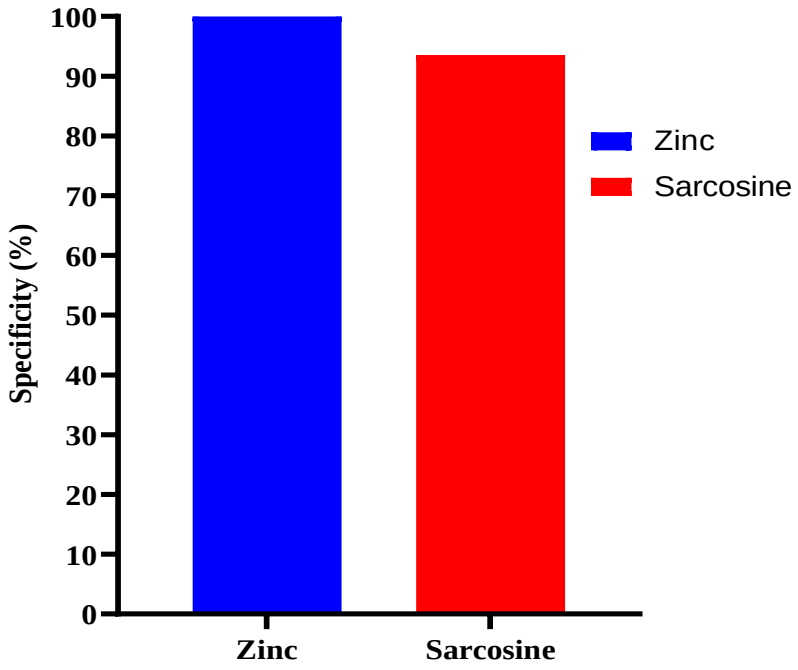

Figure 4.2: Specificity of zinc and sarcosine in testing of prostate cancer using Calorimeter and ELISA techniques

4.5 Predictive values zinc and sarcosine in diagnosis of prostate cancer using calorimetric and ELISA methods, respectively

The fourth objective was establishing the predictability of sarcosine and zinc to prostate carcinoma diagnosis. In this study a reference standard cutoff of 0.1-3µmol/ml and 1-8nmol/ml was used to determine predictive values of zinc and sarcosine respectively. In the diagnosis of prostate cancer, zinc had a 100% positive predictive value and a 93.9% negative predictive value. On the other hand, sarcosine had ppv predictive and npv values

of 93.9% and 100%, respectively, using ELISA (Figure 4.5). The probability of study participants having cancer of the prostate is high with both procedures.

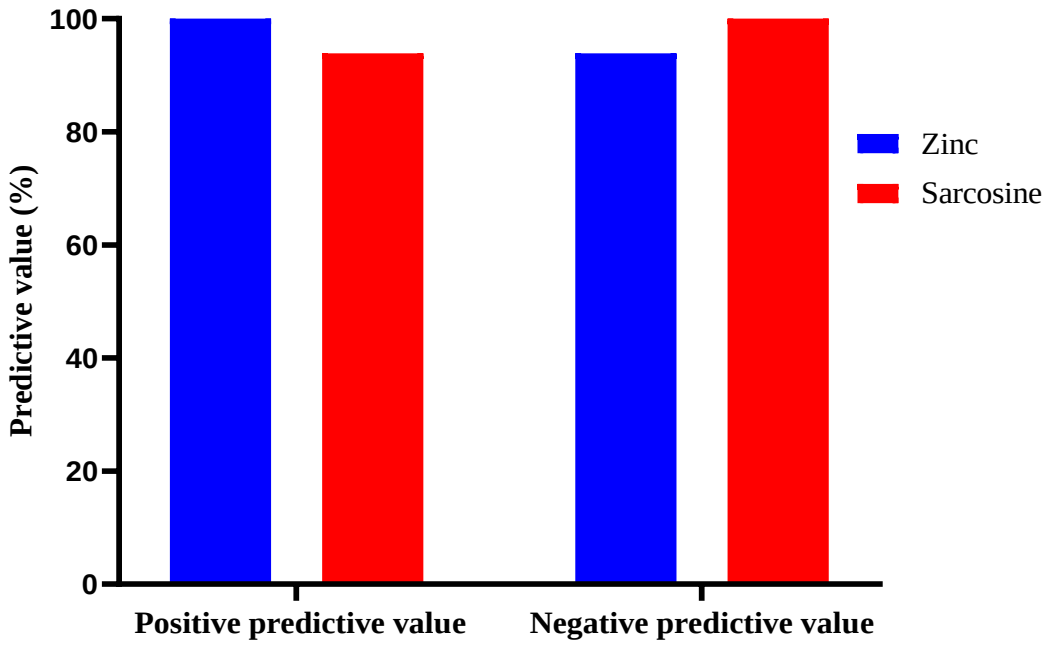

Figure 4.3: Predictive values zinc and sarcosine in diagnosis of prostate cancer using calorimetric and ELISA methods, respectively

CHAPTER FIVE: DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

The average age of participants for prostate cancer study was 66.81. The mean age was slightly lower than another study done in Europe by maxwell *et al*.,2020 whose, mean age of study participants was 71.5 years. The current study on diagnostic predictors of prostate cancer among patients attending Garissa County referral hospital is also lower in terms of mean age when compared with another study done by Cheng *et al.*,2013 in Senegal whose mean age was 71.4%.

The age bracket of 60-69 had a high percentage of participants at 32.3% followed by less than 60 years at 30.6% greater than 80 years (19.4%) and 70-79 years at 17.7%. This difference can be attributed to the vastness of the county with poor means of transport, poor road networks and lack of enough facilities for testing prostate carcinoma.

The above age group of 60-69 years with the highest number of participants correlates with a cross sectional study done on early detection of prostate cancer where 60-69 years of age had the highest number of study units (Soares *et al*.,2019).

The above trend in the existing research can be ascribed to the long distances covered to reach the testing facility the only public facility in the county of Garissa hence a low turn up with people of old age ie.70-79 years and >80 years.

Limited resources are also another challenge since most residents of Garissa County are nomads and depend entirely on cattle and other animals for their livelihood. As a result of perennial droughts, most have been rendered poorest since many animals have died.

5.1.1 Variability of zinc (calorimetric) and Sarcosine in ELISA diagnosis

Eskra *et al*.,2019 identified a high association between the output of secreted zinc from the prostate tissue and prostate carcinoma signifying that, a reduced concentration of zinc in urine a characteristic biochemical marker of prostate cancer. The current study coincides with the above study since the mean zinc concentration of control participants was significantly higher at 6.20umol/ml than the mean concentration of prostate cancer patients (0.98umol/ml) p<0. 001. However, this study is closely related to other findings by Catalona *et al.,*2012 that found out that reduced zinc levels could be used for testing cancer of the prostate. The study also correlates with a descriptive case control study done in Nigeria among prostate cancer patients and controls which noted a 69% zinc concentration in control subjects and 21.8% zinc concentration in prostate cancer patients p<0.001 (Amali *et al*., 2020).

The above findings contradict with the study hypothesis that there is no significance difference in urinary zinc levels between confirmed prostate cancer patients and those not confirmed. The study confirms the presence of low mean concentration of zinc (0.98umol/ml) with prostate cancer patients compared to control subjects whose mean zinc concentration is 6.20nmol/ml and therefore confirming a study by Catalona *et*

al.,2012 that low concentration of zinc in urine is a predictor metabolite for malignancy of the prostate.

As per the findings of the calorimetric method, the age group of prostate cancer patients showed no significant difference in urine zinc concentration p=0.85. Amali &Aleme 2020 in Nigeria observed that there was no significant difference in the concentration of zinc in urine of prostate cancer patients and controls 69.73±73 vs 68.97± 7.32. The age groups of <60,60-69,70-79, and ≥80 had zinc concentrations of 1.02±0.36,1.15±0.27, and 0.90±0.23 umol/ml respectively.

Concentration of zinc among control participants demonstrated no significant difference p=0.11. The age groups of <60,60-69,70-79 and ≥80 had zinc concentrations of 6.36±0.40,5.25±0.25,6.58±0.93 and 8.93±0.000umol/ml respectively. The means and standard error of means were not significant using one-way Anova. The study findings correlate with a cross-sectional study done on prostate cancer patients and controls in Elsevier Journal by Martynko *et al*., 2020.This study involved use of Elisa kit and noted a statistically significant difference in sarcosine and zinc concentrations in urine between the two groups i.e., Prostate cancer and non-prostate group.

The mean concentration of sarcosine in prostate cancer participants were significantly higher at 4.30 nmol/ml than those of control participants (0.47nmol/ml in ELISA diagnosis p<0.001.

The study compares with another study done by Jentzmik *et al* (2010) where sarcosine concentration levels in urine were higher when compared with control participants. A research done by Sironi *et al*.,2018 uncovered that, the mean concentration of sarcosine in urine was higher in prostate malignant participants than those not confirmed for prostate cancer ($P<0.001$).

The above study also correlates with the results in the existing research that the concentration means of sarcosine in urine are significantly higher in prostate cancer patients than those found in controls.

Jiang *et al*.,2010 discovered a new method for analyzing sarcosine metabolite using GC-MS (Gas chromatography and Mass spectrophotometry. The study noted average sarcosine concentration were raised in prostate malignant patients compared to the control group.

The hypothesis that there is no significant difference in urinary sarcosine concentration levels between confirmed prostate cancer patients and those not confirmed was rejected since there was a significant difference hence confirming that sarcosine in urine isa potential biochemical for diagnosis of prostate carcinoma. The discovery that prostate carcinoma participants had higher concentrations of sarcosine than controls lends credence to this.

The age groups of prostate cancer patients and sarcosine concentration in ELISA diagnosis had no significant difference p=0.57. The age groups of <60,60-69,70-79 and ≥80 had sarcosine concentrations of 4.30 ±0.39,4.51±0.13, 4.30±0.28, 4.23±0.19 respectively.

The above study differs with a research done by Makni *et al*.,2021 using electrochemical sensors. The current study confirms the need to seek an alternative for PSA in the screening of prostate malignancy by use of urine sarcosine as biomarker for prostate cancer diagnosis. The means and standard error of means in control participants were significantly not different (P>0.05).

Gkotsos *et al*.,2017 noted in their findings that there was no significant difference among the age groups of prostate cancer and controls. There was no correlation between sarcosine concentrations and age.

5.1.2 Sensitivity and specificity of zinc (calorimetric) and sarcosine (ELISA) in screening for prostate cancer

There was significant variation between the reality (gold standard) and zinc calorimetric diagnosis among participants in this study (P<0.001. These findings correlate with a study done in Chicago on zinc concentration in urine as a potential novel biomarker for early diagnosis of prostate cancer (Katafigiotis *et al.*,2012).

Katafigiotis *et al*.,2012 study showed a significant sensitivity of 78.6% while maintaining a specificity of 60%, P<0.001

There was a significant difference in sensitivity and specificity between this study and the current study findings whose sensitivity on prostate cancer, calorimetric diagnosis for zinc concentration in urine was 93.9% while specificity was 100%. Another study described by Madarova *et al* (2014) on prostate carcinoma participants and controls, ZPPI titration in EPS urine showed zinc concentration higher in negative cancer groups.

A prospective study on zinc as a urinary biomarker of prostate cancer in international journal of urology showed a sensitivity of 89% and 51% specificity (Theodorescu *et al.*,2008). These findings were significantly lower compared to the current study using calorimetric diagnosis.

A study by Tervana *et al*.,2018 developed a validated GC/MS procedures for analysis of sarcosine concentration in urine for prostate cancer patients and non-prostate controls. The outcome demonstrated that, sarcosine is a potential prostate tumor biomarker with a sensitivity of 79% and specificity of 87%.

The current research shows a significantly higher specificity and sensitivity of 100% and 93.9% respectively with Elisa diagnosis. This means that, adopting Elisa method in screening for prostate cancer in urine minimizes chances of false positives and negatives. According to a study by Pacik et al. (2018), the highest concentration of sarcosine (10 µmol/l) could be detected in urine with a performance of 93.5%, specificity of 91.6%,

and precision of 90%. These findings comparable to the current study which recorded a slightly higher specificity and sensitivity of 100% and 93.9% respectively.

Another study on sensitivity and specificity of sarcosine in urine by Pacik et al 2018 showed a sensitivity of 92.11%, specificity of 75% and accuracy of 89.3%. These values are not far from the findings and therefore sarcosine can be a good diagnostic tool and a marker for cancer of the prostate.

5.1.3 Predictability of zinc and sarcosine to prostate carcinoma diagnosis by calorimetric and ELISA methods respectively

ELISA and calorimetric tests of sarcosine and zinc demonstrated a significant difference with the confirmatory test in this study $P<0.001$.

Calorimetric assay for zinc showed a negative predictive value and a positive predictive value of 93.5% and 100% respectively. A study done on novel expressed prostate secretion of zinc in urine for prostate cancer diagnosis had a positive and negative predictive value of 100% and 87.5 % respectively (Drago *et al*.,2021).

These values are close to the ones of the current study whose positive and negative predictive values for sarcosine in prostate cancer diagnosis is 100% and 93.7% respectively. Generally, only few studies used ELISA and calorimetric methods in the screening of both sarcosine and zinc respectively.

Western blot and immunohistochemistry were also performed on zinc in urine on mean age of 65.7±8.7 years of prostate cancer and non-prostate cancer controls showing a significant predictive value at $p \leq 0.05$. This predictive ability was slightly lower than that of the current study using calorimetric and Elisa diagnosis. This difference could be attributed to the methods of analysis which are significantly different. (Katafigiotis *et al*.,2012) The current study using calorimetric and ELISA methods demonstrated high likelihood of an individual of having prostate carcinoma when biochemically tested for zinc or sarcosine in urine. This therefore implies that if the calorimetric and ELISA methods of diagnosis are adopted, they will be able to pick accurately the true positives and true negatives.

5.2 Conclusions

There was significantly higher zinc mean concentration of control participants than that of prostate carcinoma patients with calorimetric method.

The concentrations of sarcosine in prostate cancer participants were significantly higher than those of control participants in ELISA diagnosis

There was significant variation between reality on both sarcosine and zinc on ELISA and calorimetric diagnosis demonstrated by the values in sensitivity, specificity and predictability.

5.3 Recommendations

1. The national government and county governments need to develop policies on diagnosis of prostate cancer by using calorimetric and ELISA methods for zinc and sarcosine respectively.
2. County governments need to allocate funds for diagnostic facilities to ease the burden of diagnosis.
3. The county Government need to mobilize resources to ensure support and sustainability of prostate diagnosis at all level of facilities

5.3.1 Recommendations for further studies

1. Future studies should be done to incooperate sarcosine and zinc metabolite in urine.
2. Future researchers should investigate other metabolites to supplement zinc and sarcosine in the diagnosis of prostate cancer.

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APPENDICES

Appendix I: Informed Consent Form

My name is Januaris Mwanthi Mukaa. I am a student taking masters course in the school of medicine, Kenyatta University. Further, am permitted to conduct a study titled "Diagnostic predictors of prostate cancer among patients attending Garissa County Referral Hospital, Kenya."

The aim of this study is to investigate predictors of prostate carcinoma in urine of confirmed prostate positive and negative participants. The results of urinary sarcosine and zinc will be compared with the results of histology to determine the predictability of cancer status using urine tests.

To qualify in participating in this study, one will be required to allow information contained in his or her request formed to be used by the researcher. You will also be required to allow the researcher use the results from histology whether positive or negative. Urine specimen (30mls) will be taken from you for further tests.

Voluntarism

You will not fail to get the services from this clinic if you decline to participate. Further, the decision you take pertaining the study will not make you fail to get the care that is required. You have all the rights to voluntarily agree to the above but remember being part of this study is voluntarily. You have the freedom to ask any questions related to the study. Participation in this study may extend the number of hours of waiting in the facility

Discomforts and Risks. The study being carried out doesn't pose any risk to the participant. There are no uncomfortable procedures to be carried**.**

Benefits. Participation in this study, will help us to learn how to provide early diagnosis of prostate carcinoma before it spreads to other tissues hence proper treatment.

Reward

You will not be paid for participating in this study.

Confidentiality

Your personal data will not be put in this consent form. Every data obtained in this study will be private, and only to be shared with the study team. The study materials will be kept safe and locked at Kenyatta University.

Contact Information

If you have questions about the study call, Dr Wachuka Njoroge 0722737669 or Dr. Radol Omondi 0721398195., my contact number is 0729593968.

However, you can email the Kenyatta University Ethical Review Committee Secretariat at chairman.kuerc@ku.ac.ke if you have any questions regarding your rights as a study participant.

Participant's statement

I am aware of the details mentioned above regarding my role in the study. I've received a thorough explanation of the study, an opportunity to ask questions, and adequate responses to my inquiries. I voluntarily decide to participate fully in this study. I understand that my records will be kept private and that I can leave the study at any time. I understand that my decision will have no bearing on the care I receive from this clinic going forward or from any other clinic, regardless of whether I decide to withdraw from the study or not.

Name of Participant: _____

_____ _____

Signature or Thumbprint Date

_____ _____

Name of Representative/Witness (where necessary) Relationship to Subject

Investigators statement

The volunteer has been informed about the study's procedures, risks, and benefits in a language that they can understand by me, the undersigned.

Name of Interviewer _____ Signature_____

Appendix II: Materials and Reagents for Sarcosine Analysis

- Standard solution(16nmols/ml)
- Precoated ELISA plates(clear)
- Streptavidin-HRP
- Acidic stop solution
- Substrate solution A
- Substrate solution B
- Was buffer concentrate
- Sarcosine antibody
- Plate sealers
- 37 degrees centigrade ±0.5 incubator
- Absorbent papers
- Precision pipettes
- Disposable pipette tips
- Chemical hood
- Gloves
- Lab coat

Appendix III: Materials and Reagents for Zinc Analysis

- Zinc detector
- Assay buffer
- Zinc chloride standard
- 96 clear well plates
- Clean Pipette tips
- Clean multichannel pippete
- Eppendorf tubes(1.5ml)
- Mark pen
- Precision pipettes
- Disposable pippete tips
- Distilled water
- User instruction
- Absorbent papers
- Microreader with 485nm wavelength filters
- Plate sealers
- 37 degrees centigrade ±0.5 degrees centigrade
- Chemical hood
- Gloves
- Lab coat

Appendix IV: Comparison between PSA diagnosis and ELISA/Calorimetric diagnosis of sarcosine and zinc in urine

SAMPLE(BLOOD)	SAMPLE(URINE)
✓ Comes from virtually all body organs	✓ Comes from urogenital organs (doesn't cross blood tissue barriers)
✓ Materials from blood have interferences because of its distribution to many organs	✓ Contains substances secreted from the prostate gland without interferences
✓ Collection of blood is invasive	✓ Collection of urine is non invasive
✓ Needs more processing procedures	✓ Biochemical analysis of urine needs less processing procedures
✓ Collection of blood may lead to hemolysis	✓ Collection is easy and no hemolysis
✓ Repetitive procedures are not possible	✓ Repetitive sampling procedures possible since urine can be obtained at frequent intervals without injuring the patient.
✓ Not possible to do mass screening since most people fear venipuncture	✓ It's easy to collect during mass screening
✓ Blood contains many interfering metabolites hence not possible to get a sample which can mirror the health of prostate gland	✓ Anatomical proximity of the prostate gland to the urethra and bladder makes it possible to collect materials from prostate in urine which mirrors the health of the prostate tissue.
✓ PSA is nonspecific hence contributing to overdiagnosis and over treatment since it's not exclusive of BPH, prostatitis and exercise	✓ ELISA diagnosis of sarcosine and calorimetric zinc in urine is more specific to the prostate tissue

Appendix V: KEMRI Letter

In Search of Better Health

KENYA MEDICAL RESEARCH INSTITUTE

OFFICE OF THE DIRECTOR CORPORATE SERVICES

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P.O. Box 54840-00200, Nairobi Email: corporateservices@kemri.go.ke Website: www.kemri.go.ke

KEMRI/RD/RDKM/7/48 **28th June, 2022**

Januaris Mwanthi Mukaa
Kenyatta University
P.O BOX 43844-00100
Email : mukaaj68@gmail.com

RE: MSc PROJECT RESEARCH ATTACHMENT

Reference is made to your letter on the above mentioned subject.

We are pleased to inform you that the Institute has offered you a MSc. Research attachment at the Centre for Traditional Medicine and Drug Research (CTMDR) at Kenya Medical Research Institute, (KEMRI) for Six months with effect from **4th July 2022 to 3rd January,2023.**

We note that you will work within the Centre under the supervision of Dr. Jeremiah Gathirwa as you execute your study in '*Determination of diagonistic predictors of prostate cancer among patients attending Garissa County Refferal Hospital ,Kenya*'

However, you are required to pay a bench fee of **Kshs. 6,000** to the KEMRI A/c No. **1104174529; A**/c Name: **KEMRI;** Bank: **Kenya Commercial Bank, Kipande House Branch,** before starting the attachment and present the deposit slip to the Cash office and payment receipt to the Training office to facilitate your deployment. You will also cater for the cost of consumables for your research.

We wish you all the best in your research.

F. Rowland Muyeshi
For: DIRECTOR GENERAL & CEO
KENYA MEDICAL RESEARCH INSTITUTE
Cc: Deputy Director – CTMDR

Appendix VI: Approval by Kenyatta University Ethics Review Committee

KENYATTA UNIVERSITY
CENTRE FOR RESEARCH ETHICS AND SAFETY

Fax: 8711242/8711575
Email: chairman.kuerc@ku.ac.ke
Nairobi, 00100

P. O. Box 43844,

Tel: 8710901/12

Website: www.ku.ac.ke

Our Ref: **KU/ERC/APPROVAL/VOL.1**

Date: 20th /01/2022

Januaris Mwati Mukaa
P.O BOX 43844-00100
Nairobi.

Dear Ms. Mukaa,

APPLICATION NUMBER: PKU/2427/I1561 – DETERMINATION OF DIAGNOSTIC PREDICTORS OF PROSTATE CANCER PATIENTS ATTENDING GARISSA COUNTY REFERRAL HOSPITAL, KENYA

This is to inform you that ***KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE*** has reviewed and approved your above research proposal. Your application approval number is **PKU/2427/I1561.** The approval period is **20th *01/2022 to 20th/01/2023.***

This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, MTA) will be used
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by ***KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE***
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to ***KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE*** within 72 hours of notification
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to ***KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE*** within 72 hours
v. Clearance for export of biological specimens must be obtained from relevant institutions.

vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.

vii. Submission of an executive summary report within 90 days upon completion of the study to ***KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE***

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://research-portal.nacosti.go.ke and also obtain other clearances needed.

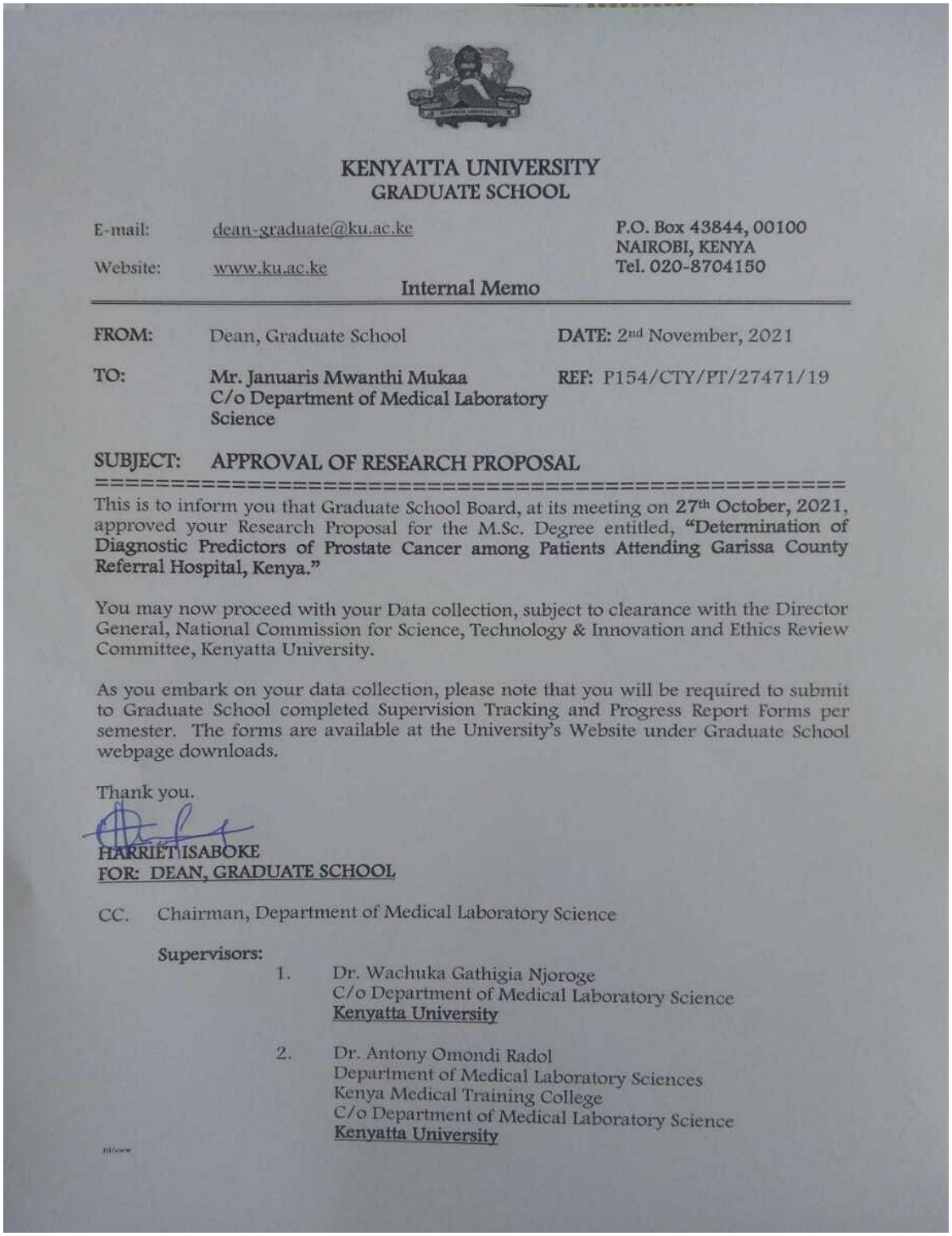
To serve you better, researchers are kindly requested to access and complete a customer feedback form and sent it back online as you continue with research and upon completion of data collection found on the following website link; ;(https://docs.google.com/forms/d/1ytWefDwvyz5h1oz_VIn0xbxg3uGdlDzMXFWNDsMrRPQ/edit?usp=sharing

Yours sincerely

Prof. Judith Kimiywe

Director: Centre for Research Ethics and Safety

Appendix VII: Research Approval



KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: dean-graduate@ku.ac.ke
Website: www.ku.ac.ke
P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 020-8704150

Internal Memo

FROM: Dean, Graduate School
DATE: 2nd November, 2021

TO: Mr. Januaris Mwanthi Mukaa
C/o Department of Medical Laboratory Science
REF: P154/CTY/PT/27471/19

SUBJECT: APPROVAL OF RESEARCH PROPOSAL

This is to inform you that Graduate School Board, at its meeting on 27th October, 2021, approved your Research Proposal for the M.Sc. Degree entitled, **"Determination of Diagnostic Predictors of Prostate Cancer among Patients Attending Garissa County Referral Hospital, Kenya."**

You may now proceed with your Data collection, subject to clearance with the Director General, National Commission for Science, Technology & Innovation and Ethics Review Committee, Kenyatta University.

As you embark on your data collection, please note that you will be required to submit to Graduate School completed Supervision Tracking and Progress Report Forms per semester. The forms are available at the University's Website under Graduate School webpage downloads.

Thank you.

HARRIET ISABOKE
FOR: DEAN, GRADUATE SCHOOL

CC. Chairman, Department of Medical Laboratory Science

Supervisors:

1. Dr. Wachuka Gathigia Njoroge
   C/o Department of Medical Laboratory Science
   Kenyatta University
2. Dr. Antony Omondi Radol
   Department of Medical Laboratory Sciences
   Kenya Medical Training College
   C/o Department of Medical Laboratory Science
   Kenyatta University

Appendix VIII: NACOSTI Research Permit

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY & INNOVATION

Ref No: **212334**

Date of Issue: **31/January/2022**

RESEARCH LICENSE

This is to Certify that Mr.. JANUARIS MWANTHI MUKAA of Kenyatta University, has been licensed to conduct research in Garissa on the topic: DETERMINATION OF DIAGNOSTIC PREDICTORS OF PROSTATE CANCER AMONG PATIENTS ATTENDING GARISSA COUNTY REFERRAL HOSPITAL for the period ending : 31/January/2023.

License No: **NACOSTI/P/22/15426**

212334

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.